

UNITED STATES DEPARTMENT OF AGRICULTURE  
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO.  
84-R-0072

CUSTOMER NO.  
26904

FORM APPROVED  
OMB NO. 0579-0036

# ANNUAL REPORT OF RESEARCH FACILITY (TYPE OR PRINT)

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA, include Zip Code)

PRINCIPAL RESEARCH SERVICES, INC.  
(b)(2)High, (b)(7)(F) PO Box 138  
FORT COLLINS, CO (b)(2)High, (b)(7)(F)  
(970) 232-1122 50522

3. REPORTING FACILITY  
(sheets if necessary.)

(b)(2)High, (b)(7)(F)

aching, or experimentation, or held for these purposes. Attach additional

See Attached Listing

FACILITY LOCATIONS (sites)

## REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain- relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)	F. TOTAL NO. OF ANIMALS  (Cols. C + D + E)
4. Dogs	0	16	170	0	176
5. Cats	0	0	0	0	0
6. Guinea Pigs	0	0	0	0	0
7. Hamsters	0	0	0	0	0
8. Rabbits	0	0	0	0	0
9. Non-Human Primates	0	0	0	0	0
10. Sheep	0	0	0	0	0
11. Pigs	0	86	139	15	240
12. Other Farm Animals	0	0	0	0	0
13. Other Animals	0	0	0	0	0

### ASSURANCE STATEMENTS

- 1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all the exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- 4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

### CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL (Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (Type or Print)

DATE SIGNED

(b)(6),(b)(7)(c)

(b)(6),(b)(7)(c)

11/26/07

(AUG 1)

FACILITY - HEADQUARTERS

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## Column E Explanation

1. **Registration Number:** 84-R-0072
2. **Number of animals used in this study:** 15
3. **Species of animals used in this study:** Pigs
4. **Explain the procedure producing pain or distress:**

For this study joint inflammation was induced by doing an intra-articular injection of a bacterial endotoxin, lipopolysaccharide (LPS), into one knee joint of the pig. This LPS model of joint inflammation has been reported in the dog but not in the pig so the model must be established in the pig since the pig will then be used for studies testing dermal absorption of anti-inflammatory drugs targeted to control joint inflammation/arthritis. The reason pigs are required for this model is because the skin of the pig is so similar in anatomy, thickness and has the ability to absorb chemicals in a way that is very similar to humans.

To establish this model one stifle joint was injected in each of 15 pigs with 10 nanograms of LPS in saline into the joint space. We then euthanized 3 pigs each at 2, 4, 8, 12 and 24 hrs after LPS injection and collect blood, synovial fluid, synovial tissue and meniscal cartilage. The joint fluid and tissues were analyzed for the concentration of cytokines which are chemicals released into the fluid and tissue in response to inflammation. The concentrations of cytokines in tissue and fluid collected at these various time points, will establish a baseline of untreated inflammatory response to LPS over 24 hours.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

Intervention with analgesic drugs will not be possible since non-steroidal anti-inflammatory drugs are known to modify prostaglandins and other cytokines involved in inflammation. Likewise opioid analgesics affect macrophages and other inflammatory cells and would be a confounding variable in the study since these cells are integral to the development of inflammation.

A PubMed literature search was done to confirm the statement above regarding opioid analgesics affect on inflammation. The following references are examples of many publications that confirm this.

Malik AA, Radhakrishnan N, Reddy K, Smith AD, Singhal PC.

Morphine-induced macrophage apoptosis modulates migration of macrophages: use of in vitro model of urinary tract infection. J Endourol. 2002 Oct;16(8):605-10.

Azuma Y, Ohura K.

Endomorphin-2 modulates productions of TNF-alpha, IL-1beta, IL-10, and IL-12, and alters functions related to innate immune of macrophages. Inflammation. 2002 Oct;26(5):223-32.

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Bhaskaran M, Reddy K, Sharma S, Singh J, Radhakrishnan N, Kapasi A, Singhal PC.  
Morphine-induced degradation of the host defense barrier: role of macrophage injury.  
J Infect Dis. 2001 Dec 15;184(12):1524-31. Epub 2001 Dec 3.

Roy S, Charboneau RG, Barke RA.  
Morphine synergizes with lipopolysaccharide in a chronic endotoxemia model.  
J Neuroimmunol. 1999 Mar 1;95(1-2):107-14.

**Note on experimental design refinement after this study was completed.**

After this study was completed the results were that the maximum inflammatory response to LPS injection was seen within 2 hours of LPS injection. Therefore in subsequent studies using LPS joint injection, we were able to shorten the study to 2 hours instead of going out to as long as 24 hours after LPS injection. In addition, all pigs were maintained under injectable Telazol anesthesia throughout this 2 hour period. Therefore USDA pain category for subsequent studies was changed to category D.